Remarks

In response to the telephone conversation between Patrea Pabst and Richard Hudson, the claims have been amended, as suggested, to correspond to the claims as amended in the Amendment and Response to Restriction Requirement dated October 21, 2004, with a minor modification noted below. These amendments are made with the understanding that claims 4, 5, 7-12, 16-19, 23-26 and 29 are no longer withdrawn and that all of the claims will now be examined together on the merits.

Independent claims 1, 13, and 20 have also been amended to clarify that the medium chain length PHAs are accumulated through the fatty acid biosynthesis pathway. These amendments do not contain new matter. The amendments are made by reference to Figure 1 and the accompanying description in the specification.

As discussed below with regard to the written description and enablement rejections, it has been known since 1989 that one could engineer bacteria or plants with genes encoding one or more of the enzymes in the traditional polyhydroxyalkanoate ("PHA") biosynthetic pathway (see right hand side of Figure 1, referring to the reductase, thiolase and PHA synthase). Genes encoding these enzymes are well known from any different sources and have been shown to be transferable into bacteria or plants to make PHA. See pages 1 to 3 of the application as originally filed.

What applicants have developed and claimed is how to make medium chain (defined in Figure 1 as where x=4 or 6, for example) PHAs accumulate through the fatty acid biosynthetic pathway (shown in Figure 1 on the left side). As is clear from Figure 1, there are three pathways

from the fatty acids to medium chain length PHAs. All pathways start with the fatty acids, all are polymerized by the medium chain length PHA synthase. The alternative pathways are shown as (1) A; (2) B to C; (3) B to D. This application is directed to the latter two, both of which use B, 3 hydroxyacyl-ACP thioesterase. C is the acyl CoA synthase; D is the acyl CoA transferase. Accordingly, since simply referring to the production of polyhydroxyalkanoate could confuse which pathway is being utilized, the claims are now drawn to the medium chain PHA pathway, beginning with fatty acid biosynthesis. The enzymes that are essential are the 3 hydroxyacyl-ACP thioesterase and the medium chain length PHA synthase, and either the acyl Co synthetase or the acyl CoA transferase. Any of these can be provided in the form of a transgene.

Rejection Under 35 U.S.C. § 112, first paragraph

Claims 1-3, 6, 13-15 and 20-22 were rejected under 35 U.S.C. § 112, first paragraph, as allegedly lacking written description on the basis that the specification contained only one DNA sequence encoding 3-hydroxyacyl-ACP thioesterase and only one DNA sequence encoding acyl CoA synthetase. Applicants respectfully traverse this rejection.

The written description requirement for a claimed genus may be satisfied through a description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant identifying characteristics, i.e. structure or other physical and/or chemical properties, by functional characteristics coupled with a known or a disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus (See *Regents of Univ. of Cal. v. Eli Lilly & Co.*, 119 F.3d 1559, 1564 (Fed. Cir., 1997)).

The specification clearly discloses the relevant identifying characteristics of the claimed genus by function, i.e. genes encoding proteins with 3-hydroxy-ACP thioesterase activity, acyl CoA synthetase activity, or acyl CoA transferase activity. Genes encoding these activities were known at the time this application was filed, as well as methods to screen for genes encoding these activities or other activities which were routine for one of ordinary skill in the art.

3-hydroxy-ACP thioesterase

Specifically, genes encoding proteins with 3-hydroxy-ACP thioesterase activity are described in the specification at least at page 2, lines 24-30 and were known in the art. See for example, Rehm, et al., *J. Biol. Chem.* 273:24044-24051 (1998) ("Rehm"), U.S. Patent No. 5,750,848 to Kruger, et al. ("Kruger"), and Hoffmann, et al., *FEMS Microbiology Letters* 184:253-259 (2000) ("Hoffmann"), submitted in the Information Disclosure Statement (IDS) filed May 8, 2002. Rehm and Kruger disclose the *PhaG* gene from *Pseudomonas putida* (see page 24044 of Rehm and column 2-3 of Kruger). Rehm disclose that *phaG* catalyzes the conversion of 3-hydroxyacyl-ACP to 3-hydroxyacyl-CoA derivatives (see page 24050 column 1). Hoffmann discloses the *PhaG* gene from *Pseudomonas aeruginosa* and that this enzyme exhibits 3-hydroxydecanoyl-CoA-ACP transacylase activity (see page 253, abstract). 3-hydroxy-ACP thioesterase activity is referenced in pathway B of Figure 1 in the present application. Screening methods to isolate an enzyme or combination of enzymes that allow conversion of 3-hydroxy acyl ACPs to 3-hydroxy acyl CoAs (i.e. an enzyme with 3-hydroxyacyl-ACP thioesterase activity) in PHA negative bacteria were also known in the art, as demonstrated in Kruger (see column 11), and described in the specification at least at page 6,

lines 20-24. Methods for identifying genes that encode 3-hydroxy acyl ACP thioesterase activity are also disclosed in the specification at least at page 10, line 27 to page 11, line 12. The specification at least at page 11, lines 10-13 disclose identifying genes encoding 3-hydroxyacyl-ACP thioesterase activity by testing libraries of genes for complementation of *phaG* mutant strains or in heterologous bacteria expressing a suitable PHA synthase and a 3-hydroxyacyl-CoA synthetase. Identification of genes by complementation was well known to one of ordinary skill in the art. See for example, the enclosed abstracts demonstrating identification of genes by complementation including PHA synthases (Nishikawa, et al., *Curr. Microbiol.* 44(2):132-135 (2002)), PHA synthases and acetoacetyl-CoA reductases (Umeda, et al., *Appl. Biochem. Biotechnol.* 70-72:341-352 (1998)), and other genes involved in PHA synthesis (Liebergesell and Steinbuchel, *Appl. Microbiol. Biotechnol.* 38(4):493-501 (1993)).

Acyl CoA synthetase

Acyl CoA synthetase activity is referenced in pathway D of Figure 1 of the specification. Genes encoding acyl CoA synthetase activity are disclosed in the specification at least at page 7, lines 12-18 and were well known to those skilled in the art. See for example Black, et al., *J. Biol. Chem.* 267:25513-25520 (1992) ("Black"), van Beilen, et al., *Molecular Microbiology* 6:3121-3136 (1992) ("van Beilen"); and Matesanz, et al., *J. Mol. Biol.* 291:59-70 (1999) ("Matesanz"), submitted in the IDS filed May 8, 2002. Black discloses the *fadD* gene of *Escherichia coli* encoding acyl coenzyme A synthetase activity (see page 25513, abstract). Van Beilen discloses *alkK* gene of *Pseudomonas olevorans* encoding acyl coenzyme A synthetase activity (see page 3121, abstract). Matesanz discloses the *pfacs1* gene from *Plasmodium*

falciparum encoding acyl coenzyme A synthetase activity (see page 59, abstract). As described above, methods for identifying genes via complementation, including genes encoding acyl CoA synthetase activity are well known to those skilled in the art and are disclosed in the specification at least at page 8, lines 18 to page 9, line 6.

Acyl CoA transferase

Acyl CoA transferase activity is shown in pathway D in Figure 1. Genes encoding acyl CoA transferase activity are disclosed in the specification at least at page 8, line 2-4, and were well known to those skilled in the art. See for example, Madison and Huisman, *Microbiol. Mol. Bio. Rev.* 63(1):21-53 (1999) ("Madison") and Sohling, et al., *J. Bacteriol.* 178:871-880 (1996) ("Sohling"), submitted in the IDS filed May 8, 2002. Sohling discloses the *cat1* gene encoding acyl CoA transferase activity from *Clostridium kluyveri* (see page 871, abstract). Sohling also states on page 878, column 1, that CoA transferases from *Pseudomonas putida, Acinetobacter calcoaceticus, Clostridium acetobutylicum*, and from the pig heart have been identified. Madison reviews the pathways involved in engineering PHAs. Madison discloses genes encoding acyl CoA transferase activity including the *hbcT* gene from *Clostridium kluyveri* (see also Figure 4). Methods to identify genes encoding acyl CoA transferase activity are disclosed in the specification at least at page 7, line 28, to page 8, line 9 and methods for identifying genes via complementation, including genes encoding acyl CoA synthetase activity are well known to those skilled in the art (for examples of other methods see Sohling submitted in the IDS filed May 8, 2002).

PHA Synthases

PHA synthases and methods to identify genes encoding PHA synthase activity were well known to one of ordinary skill in the art and are disclosed in the specification at least at page 9, lines 7-9. Genes encoding PHA synthases are also disclosed in the specification at least at page 2, lines 5-23 and were known to one of ordinary skill in the art. See for example, Huisman, et al., *The Journal of Biological Chemistry* 266(4):2191-2198 (1991) ("Huisman"), Madison and Huisman, *Microbiol. Mol. Bio. Rev.* 63(1):21-53 (1999) ("Madison"), and U.S. Patent 5,250,430 to Peoples, et al., ("Peoples"), submitted in the IDS filed May 8, 2002. Madison discloses genes encoding PHA synthases including genes from *R. eutropha, P. oleovorans, and Synechocystis sp.* (see Figure 2). Huisman discloses genes encoding PHA synthases from *Pseudomonas oleovorans* (see page 2191, abstract). Peoples disclose PHA synthases from *Zoogloea ramigera, Alcaligenes eutrophus, Nocardia salmonicolur,* and *Pseudomonas oleovorans* (abstract). Methods to identify genes encoding PHA synthases are described in U.S. Patent No. 6,143,952 to Srienc, et al. ("Srienc"), submitted in the IDS filed May 8, 2002, and methods for identifying genes via complementation, including genes encoding PHA synthases are well known to those skilled in the art (see for example the enclosed abstracts Nishikawa, et al., *Curr. Microbiol.* 44(2):132-135 (2002) and Umeda, et al., *Appl. Biochem. Biotechnol.* 70-72:341-352 (1998)).

As described above, the specification clearly discloses genes encoding 3-hydroxy-ACP thioesterase activity, acyl CoA synthetase activity, and acyl CoA transferase activity and methods to identify such genes. Therefore, the written description requirement for claims 1-26 and 29 is satisfied.

Rejection Under 35 U.S.C. § 112, first paragraph, enablement

Claims 1-3, 6, 13-15 and 20-22 were rejected under 35 U.S.C. § 112, first paragraph, as not being enabled. Applicants respectfully traverse this rejection.

Genetically engineered plants and bacteria that make polyhydroxyalkanoates are known, having been described in the patent literature since 1989. The problem applicants were addressing is how to produce *high levels of medium chain length polyhydroxyalkanoates* (see page 3, line 27 to page 4, line 1 and page 6, lines 17-20). The solution, as described on page 4, lines 19-24, and page 7, line 3 to page 8, line 9, and defined by the claims, is to provide, in addition to the other enzymes for polyhydroxyalkanoate production (beta-ketothiolase, acetyl CoA reductase and PHA synthase), an acyl CoA synthetase and/or a CoA transferase.

Whether the disclosure is enabling is a legal conclusion based upon several underlying factual inquiries (See *In re Wands*, 858 F.2d 731, 735, 736-737, 8 USPQ2d 1400, 1402, 1404 (Fed. Cir.1988)). As set forth in Wands, the factors to be considered in determining whether a claimed invention is enabled throughout its scope without undue experimentation include the quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability or unpredictability of the art, and the breadth of the claims. The fact that some experimentation is necessary does not preclude enablement; what is required is that the amount of experimentation 'must not be unduly extensive' (*In re Atlas Powder Co.*, v. E.I. DuPont De Nemours & Co. , 750 F.2d 1569, 1576, 224 USPQ 409, 413 (Fed. Cir.1984)). In addition, **there is no requirement for examples.**

A proper analysis of the Wands factors shows that claims 1-3, 6, 13-15 and 20-22 satisfy the enablement requirement. The claims define a genetically engineered organism selected from the group consisting of bacteria and plants producing PHA (see page 4, lines 3-5) (which have been known since 1989), the improvement comprising providing the organism with a transgene encoding an enzyme having the catalytic activity of 3-hydroxyacyl-ACP thioesterase (see page 4, lines 6-8) so that medium chain length PHA accumulates (page 4, lines 26-27) and methods of making PHAs using the same (page 4, lines 6-11).

As discussed above, the specification clearly discloses genes encoding 3-hydroxy-ACP thioesterase activity, acyl CoA synthetase activity, and acyl CoA transferase activity and methods to identify such genes. Screening methods to isolate an enzyme or combination of enzymes that allow conversion of 3-hydroxy acyl ACPs to 3-hydroxy acyl CoAs (i.e. an enzyme with 3-hydroxyacyl-ACP thioesterase activity) in PHA negative bacteria are known in the art (see U.S. Patent No. 5,750,848 to Kruger, et al.) and described in the specification at least at page 6, lines 20-24. Methods for identifying genes that encode 3-hydroxy acyl ACP thioesterase activity are also disclosed in the specification at least at page 10, line 27 to page 11, line 12. The specification at least at page 11, lines 10-13 disclose identifying genes encoding 3-hydroxyacyl-ACP thioesterase activity by testing libraries of genes for complementation of *phaG* mutant strains or in heterologous bacteria expressing a suitable PHA synthase and a 3-hydroxyacyl-CoA synthetase. As discussed above, complementation is well known to one of ordinary skill in the art (see the enclosed abstracts, Nishikawa, et al., *Curr. Microbiol.* 44(2):132-135 (2002), Umeda,

et al., *Appl. Biochem. Biotechnol.* 70-72:341-352 (1998), and Liebergesell and Steinbuchel, *Appl. Microbiol. Biotechnol.* 38(4):493-501 (1993)).

Methods of making constructs containing genes of interest for use in plant transformation are disclosed in the specification at least at page 9, line 10 to page 10, line 10 and are well known to one of ordinary skill in the art (see for example Protrykus and Spangenberg, eds. Gene Transfer to Plants, Springer-Verlag:Berlin Heidelberg New York (1995), Maliga, et al., Methods in Plant Molecular Biology: A Laboratory Course Manual, Cold Spring Laboratory Press:New York (1995), and Owen and Pen, eds., Transgenic Plants: A Production System for Industrial and Pharmaceutical Proteins, John Wiley & Sons Ltd: England (1996), submitted in the IDS filed May 8, 2002). Methods of transforming plants with such constructs are disclosed in the specification at least at page 9, lines 11-19, and at page 15, lines 10-21 and are well known to one of ordinary skill in the art (see for example U.S. Patent No. 5,464,765 to Coffee, et al., Protrykus and Spangenberg, eds. Gene Transfer to Plants, Springer-Verlag:Berlín Heidelberg New York (1995), Maliga, et al., Methods in Plant Molecular Biology: A Laboratory Course Manual, Cold Spring Laboratory Press:New York (1995), and Owen and Pen, eds., Transgenic Plants: A Production System for Industrial and Pharmaceutical Proteins, John Wiley & Sons Ltd: England (1996), submitted in the IDS filed May 8, 2002). Methods of making bacterial constructs containing genes of interest are **extremely** routine and well known to one of ordinary skill in the art. Methods of transforming bacteria with such constructs are also **extremely** routine and well known to one of ordinary skill in the art. The specification discloses at least at page 6, lines 24-26 and at page 3, lines 1-14, PHAs can be produced by bacteria expressing transgenes

(see also for example, Rehm, et al., *J. Biol. Chem.* 273:24044-24051 (1998) and Fiedler, et al., *Applied and Environmental Microbiology* 66:2117-2124 (2000), submitted in the IDS filed May 8, 2002). As noted above the problem applicants were addressing is how to produce high levels of medium chain length polyhydroxyalkanoates and the solution as defined by the claims, is to provide, in addition to the other enzymes for polyhydroxyalkanoate production, an acyl CoA synthetase or a CoA transferase. The specification discloses at least at pages 21 to 25, bacterial constructs containing genes encoding the activities as defined by the claims and production of medium chain length PHAs. The specification clearly enables one of ordinary skill in the art to make and use the transgenic organisms as defined by the claims. Therefore, claims 1-26 and 29 are enabled by the specification.

Allowance of claims 1-26 and 29, as amended, is respectfully solicited.

Respectfully submitted,

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